Canaigre Investigations†

IV. Fermentation of Liquors for Production of High Purity Extracts*

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Introduction

Early studies of the use of canaigre for tanning demonstrated that it made good upper, saddle and fancy leathers.[3] It tanned quickly and produced soft, strong leather. The fact that these extracts contained rather large percentages of non tannins probably explains why their predominating use at that time was in the tannage of light leathers. Tanning extracts of high purity and a relatively low percentage of non tannins are generally preferred for heavy leather tanning.

* This is the fourth of a series of papers reporting various phases of cooperative investigations of canaigre as a source of tannin by the Bureau of Agricultural and Industrial Chemistry and the Bureau of Plant Industry, Soils and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, and the Texas Agricultural Experiment Station.

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There is a considerable difference in the tannin content and purity of different strains of canaigre and in lots grown in different areas. Purities range from about 40 to 55 and tannin contents from about 20 to 35 per cent on a moisture-free basis. Canaigre strains from Arizona are usually higher in tannin and purity than those from New Mexico.

For cultivation, stock would no doubt be selected which gives high root yields, high tannin content and high purity. Nevertheless, it is probable that the purity of the leach liquors would seldom greatly exceed 50. In leaching, the sugars and easily soluble non tannins are usually more quickly and completely extracted than the tannin. This sometimes results in producing leach liquors with lower purities than those shown by analysis of the original material.[2] Materials like canaigre which contain rather high percentages of non tannins, unless specially treated, normally give liquors and extracts of low purity. It is evident, therefore, that if canaigre is to be successfully used for the preparation of high purity tanning extracts, a process must be developed which will increase the purity of canaigre liquors and the extracts made from them.

The most obvious method would be to remove at least a portion of the soluble non tannins. Two procedures are available. One depends upon the difference in solubility of tannin and non tannins at temperatures just above freezing. The other depends upon the decomposition of sugars by suitable microorganisms. Considerable information has been obtained concerning the former, but it will not be reported until further work has been done. The fermentation procedure only will be discussed in this paper.

Fermentation of Canaigre Slurrys and Liquors

It would seem that a plant material containing up to 20 per cent soluble sugars, a large proportion of which is sucrose, should be easily fermented by a variety of microorganisms. The problem is complicated, however, because many microorganisms will not grow in the presence of tannin. Very little can be done to alter the compositions of canaigre liquors to make them suitable for growth of microorganisms. The addition of nutrient salts is undesirable because they would contaminate the extract; proteinaceous materials are immediately precipitated by the tannin; the pH cannot be changed except very slightly from the normal 4.0 to 5.5 because this would adversely affect the tannin; and it seems quite likely that many growth factors required by certain organisms are inactivated by tannin. Finally, the organism used must not destroy tannin.

When this work was started, the only canaigre roots available for study were those obtained from New Mexico and Texas. These were low in purity, rather low in tannin and high in sugar. Many attempts were made to ferment water extracts and slurries of these roots by a variety of microorganisms.

Most of them were known cultures which would be expected to produce valuable fermentation products that might be recovered during later processing. Among the microorganisms tried were *Bacillus macerans, Bacillus acetoaethylicus, Aerobacillus polymyxa* (two strains), *Lactobacillus delbrückii* (two strains), the anaerobes *Clostridium felsineum, Cl. acetobutylicum* (two strains) and *Cl. roseum,* four known strains of *Saccharomyces cerevisiae* and two unidentified yeasts, and one species of the mold *Rhizopus.*

None of the bacteria grew in the canaigre liquors. Some success was attained with one strain of *S. cerevisiae* (American Type Culture Collection No. 764). This organism was acclimated to canaigre liquor by gradually increasing the concentration of tannin. Fermentation was slow and incomplete, and very large inocula were required to initiate growth. Nearly theoretical yields of alcohol were obtained. Attempts to acclimate the other strains of known yeasts were not successful.

A "wild" yeast was isolated from spent canaigre which produced hyphae on solid media. This organism grew on the liquors but only decomposed a small portion of the sugars.

The *Rhizopus* species grew poorly on canaigre and canaigre liquors, and since the lactic acid, if produced in large amounts, might be almost as objectionable as sugar in the liquors, this mold was not studied further.

Since these liquors were of very low purity to start with, none of the three partially successful fermentations produced the desired results, namely, high purity liquors.

In the fall of 1943 a shipment of canaigre roots grown, shredded and sun-dried in Arizona[4] was received. Whereas the roots from Texas and New Mexico were brownish yellow, these were dark red. When some of the shredded material was placed in water and incubated at 30° C., active fermentation developed. The causative organism was isolated and the culture purified. It was a small, gram-negative rod, capable of almost completely decomposing the sugars in the Arizona roots without materially affecting the tannin.

Since that time several other bacteria have been isolated which grow well on Arizona canaigre. When grown on identical portions of canaigre liquors these bacteria exhibit quite dissimilar growth characteristics. Some produce a slight granular precipitate, some a heavy, leathery pellicle, and others a gelatinous pellicle and ropiness in the liquors. They are all small, gram-negative rods and appear to belong to the genus *Aerobacter,* although they differ somewhat from typical representatives of this group in that they ferment lactose very slowly. Four known cultures of *Aerobacter aerogenes* also grew in Arizona roots and decomposed the sugars, but some 20 cultures of the same species, isolated from egg powder, failed to grow on canaigre liquor.

With the yellow roots from Texas and New Mexico, the results were quite different. In some samples the isolates failed to grow at all; in others growth occurred, but the sugars were incompletely decomposed. The bulk lot of roots from New Mexico obtained in 1945 was fermented almost as readily as the Arizona samples, at least by some of the bacteria. A comparison of the action of these organisms on Arizona and New Mexico roots may be obtained from Table I.

In the Arizona roots, the tannin content was not appreciably affected by these bacteria, and the sugars were more completely decomposed, giving correspondingly higher purities. It should be noted, however, that the amounts of sugar decomposed were greater with the New Mexico than with the Arizona materials and that the increase in purity was actually greater with the New Mexico than with the Arizona roots. The same four organisms were the most effective ones on both strains of roots, but the order of their effectiveness was not the same. For instance, HC ranked fourth in its effectiveness on Arizona roots but was first on New Mexico roots; it was only slightly better, however, than SR2, WH and B25. When first isolated, HA was very effective on the Arizona stock; it was more active than B25. However, after both the organisms had been cultured on canaigre liquor for more than 2 years, the activity of HA had decreased markedly, whereas that of B25 had remained constant or perhaps increased.

The pH changes due to the growth of these bacteria were not usually marked but, since canaigre liquors are highly buffered, these changes probably indicate the production or destruction of considerable quantities of alkaline or acid materials, as the case may be. The same organism may produce an increase of pH with one lot of canaigre and a decrease with another, as illustrated by the action of WH and B25. This is evidence that there are fundamental difference in the strains of roots.

Just what these differences are has not been determined, but the possibility suggested itself that the strains of roots difficult to ferment or non-fermentable, contain some light labile compound inhibitory to bacterial growth and that it was altered by sun drying. To test this assumption, yellow roots which did not ferment readily were irradiated with a mercury vapor lamp to simulate sunlight. This treatment caused the roots to turn from yellow to red but did not improve their fermentability. This, plus the fact that Arizona stock transplanted and grown in New Mexico ferments equally as well as the same stock grown in Arizona, lends emphasis to the postulation that the variation in fermentability is due to differences in strains of roots.

Some indication has been obtained that an inhibitory substance is present in the non-fermentable roots but the evidence is not conclusive. This problem awaits further study.

Since the purpose of this investigation was primarily the production of

TABLE I

Action of Various Bacteria on Shredded Canaigre Roots*†

Culture No.	Source of Cultures	Total Solids	Soluble Solids	Insol-ubles	Non Tannin	Tannin	Purity	Reducing Sugars	Total Sugars	pH
		Per cent	Per cent	Per cent	Per cent	Per cent	T/SS	Per cent	Per cent	
	Arizona Roots‡									
Control		44.5	42.2	2.3	19.3	22.9	54.4	2.9	10.1	4.9
B25	Arizona roots, shredded	35.1	30.9	4.2	8.7	22.2	72.0	0.0	0.4	5.3
WH	Texas soil in which canaigre had grown	34.9	31.4	3.5	8.9	22.5	71.5	0.1	0.6	5.1
SR2	Arizona roots grown in New Mexico	35.3	31.8	3.5	9.2	22.6	70.9	0.0	0.2	4.8
HC	Leach liquor from New Mexico roots	33.6	30.7	2.9	9.2	21.5	70.0	0.0	0.4	5.9
B199	*Aerobacter aerogenes*	36.0	32.7	3.3	10.6	22.1	67.5	0.0	2.4	4.8
B562	*Aerobacter aerogenes*	38.6	35.3	3.3	12.9	22.4	63.5	0.0	4.0	4.7
RD	New Mexico roots, fresh	40.2	36.1	4.1	13.8	22.3	61.7	0.0	4.6	4.7
SR3	Arizona roots grown in New Mexico	40.2	37.2	3.0	15.2	22.0	59.2	0.1	6.0	4.9
HA	Arizona roots, shredded	40.7	38.0	2.7	16.8	21.2	55.9	0.9	8.0	4.8
	New Mexico Roots§									
Control		55.4	53.5	1.9	29.8	23.7	44.3	5.0	20.8	5.2
HC	Leach liquor from New Mexico roots	37.3	35.3	2.0	11.4	23.9	67.7	0.8	3.2	5.4
WH	Texas soil in which canaigre had grown	40.2	37.0	3.2	12.0	25.0	67.5	0.9	3.7	4.7
SR2	Arizona roots grown in New Mexico	39.1	35.6	3.5	11.8	23.8	67.0	1.1	3.8	5.0
B25	Arizona roots, shredded	40.0	36.4	3.6	12.2	24.2	66.6	0.8	3.5	5.1
SR3	Arizona roots grown in New Mexico	43.7	41.1	2.6	17.0	24.1	58.7	0.6	7.5	4.7
RD	New Mexico roots, fresh	43.8	41.2	2.6	17.0	24.2	58.7	0.6	7.3	4.7
B199	*Aerobacter aerogenes*	45.0	42.8	2.2	19.8	23.0	53.8	0.2	10.1	4.8
B562	*Aerobacter aerogenes*	53.5	50.8	2.7	27.7	23.1	45.5	2.5	18.0	4.5
HA	Arizona roots, shredded	50.6	49.1	1.5	26.8	22.3	45.4	1.1	17.9	4.5

*Extraction for tannin analysis was carried out in Reed-Churchill extractors at 65° C. Tannin analyses were made by the Official A.L.C.A. method[1].
†Results were calculated on the basis of the original moisture-free material.
‡Incubated for 66 hours at 30° C. without agitation or aeration.
§Incubated for 88 hours at 30° C. without agitation or aeration.

high-purity tanning extracts, these bacteria were not positively identified, and the products of their growth were not determined. Most of the fermentations of canaigre and canaigre liquors were carried out with the bacterium WH. This organism grows rapidly and does not produce ropy liquors or heavy pellicles.

The liquors used for fermentation and extract preparation were those obtained from the extraction studies reported previously[2] Only those obtained after the normal leaching cycle had been established were used, since they were of the approximate strength and composition that would be obtained in practice. Results of some representative tests are recorded in Table II.

Inocula of about one-tenth the volume of liquor to be fermented were prepared by seeding small amounts of the liquor from 24 hour slants and using these to seed larger volumes until the required volume was reached. Incubation was carried on at 30° C. with aeration. Under these conditions liquors from Arizona roots were almost completely fermented in 16 to 20 hours. Fermentation of liquors from New Mexico roots required about twice as long.

Canaigre liquors normally contain rather hign proportions of insolubles, which are increased somewhat by fermentation. In order to produce really high-quality extracts, they must be removed or at least reduced appreciably. This has been accomplished by centrifugal filtration of the liquors through a filter cloth thinly coated with diatomaceous earth (filter aid). This method is effective, but no doubt other equally suitable means could be found.

In Experiment 2, Table II, the liquors were divided into two equal portions. One lot was filtered and immediately evaporated to extracts. The other was fermented then filtered, after which extracts were prepared. The powdered extract from the unfermented liquor contained 49.7 per cent tannin and had a purity of 53.0; that from the fermented liquor contained 62.1 per cent tannin and had a purity of 68.4. Insolubles in each case were rather low, 3.6 and 2.8 per cent, respectively. Without filtration, the insolubles in these extracts would have been 14 to 17 per cent.

Experiment 3, Table II, illustrates the type of extract obtained when extraction was more exhaustive and was carried on at a higher temperature. Although the sugars were not completely destroyed by fermentation, their complete removal would not have resulted in as good an extract as that obtained from Experiment 2. The insolubles content was about twice as high, indicating that non-filterable high molecular weight particles had been extracted. The extract itself was cloudy and of inferior quality.

The extracts in Experiment 5, Table II, are typical of those prepared from this lot of Arizona canaigre by wet pulping disintegration and centrifugal extraction followed by fermentation and clarification. These are

TABLE II

Increase in Purity of Canaigre Liquors and Extracts by Fermentation*

Experiment No.†	Description	Total Solids	Soluble Solids	Insolubles	Non Tannin	Tannin	Purity	Total Sugars
		Per cent	Per cent	Per cent	Per cent	Per cent	T/SS	Per cent
	Composite liquors from Arizona roots							
	Untreated	4.4	3.9	0.5	1.8	2.1	53.8	0.9
	Filtered	4.1	4.0	0.1	1.9	2.1	52.5	....
	Fermented and filtered	3.2	3.1	0.1	0.9	2.2	71.0	0.1
2	Extracts from unfermented liquor							
	Liquid	51.0	49.3	1.7	23.3	26.0	52.7	...
	Powdered	97.3	93.7	3.6	44.0	49.7	53.0	22.3
	Extracts from fermented and filtered liquor							
	Liquid	31.5	30.6	0.9	9.4	21.2	69.3	...
	Powdered	93.6	90.8	2.8	28.7	62.1	68.4	0.7
	Composite liquors from Arizona roots							
	Untreated	4.9	4.3	0.6	2.2	2.1	48.8	1.2
	Fermented and filtered	3.3	3.2	0.1	1.2	2.0	62.5	0.3
3	Extracts from fermented and filtered liquor							
	Liquid	41.6	38.1	3.5	14.1	24.0	63.0	1.8
	Powdered	95.0	87.6	7.4	32.9	54.7	62.4	3.7
	Composite liquors from Arizona roots							
	Untreated	4.4	4.2	0.2	1.8	2.4	57.1	0.9
	Fermented and filtered	3.4	3.3	0.1	1.0	2.3	70.0	0.1
5	Extracts from fermented and filtered liquor							
	Liquid	44.6	43.3	1.3	13.0	30.3	70.0	...
	Powdered	95.9	92.6	3.3	27.9	64.7	69.9	0.3
	Composite liquors from New Mexico roots							
	Untreated	5.4	5.0	0.4	2.9	2.1	42.0	1.6
8	Fermented and filtered	3.3	3.2	0.1	1.3	1.9	59.4	0.5
	Extracts from fermented and filtered liquor							
	Liquid	48.2	46.4	1.8	20.1	26.3	56.7	...
	Powdered	96.7	93.0	3.7	39.6	53.4	57.4	6.5

*Tannin analyses were made by the Official A.L.C.A. method[1].

†The experiment numbers refer to the same experiments reported in Reference 2, Table III.

excellent extracts, having not more than 3.5 per cent insolubles, 65 per cent tannin and purities of about 70.

Experiment 8, Table II, is included to illustrate the results obtained with a different lot of canaigre. These extracts were prepared from a shipment of roots from New Mexico received in the fall of 1945. Analysis showed that this material had a purity of more than 50, but the liquors obtained by wet pulping and centrifugal extraction had a purity of only 42. This difference is accounted for by the fact that almost 10 per cent more non tannin is recovered than is shown by analysis of the original material. It was thought that heating the liquors to sterilize them before fermentation might have destroyed some tannin, thus causing a decrease in purity. However, tests have shown that heating these liquors at 100° C. actually increases the tannin content slightly. Although these extracts with only 53.4 per cent tannin and a purity of 57.4 were not nearly as good as those from the Arizona stock, they were comparable with many now in use and might readily be used in blends. The sugar content was rather high, but the fermentation had presumably gone to completion, and more sugar was actually decomposed than in the liquors from the Arizona stock.

Discussion

The preparation of high purity extracts from canaigre liquors involves, aside from clarification, only one step not already in use by extract manufacturers, namely, fermentation. The organisms so far found to be satisfactory for reducing the sugar content of canaigre liquors on a laboratory scale may not be suitable for large-scale fermentations or for the production of valuable by-products. These problems are being investigated, with the ultimate goal of finding a microorganism capable of effectively reducing the sugar content of canaigre liquors on a commercial scale and at the same time producing commercially valuable and easily recoverable fermentation products.

Summary and Conclusions

In order to produce high-purity extracts from canaigre liquors it is necessary to reduce the non tannin content. Attempts to do this by fermenting the liquors with some identified cultures of bacteria, yeasts and molds were unsuccessful. The tannin in tne liquors affected the microorganisms to such an extent that many failed to grow at all; others grew poorly, and decomposed only a small portion of the sugar. Several cultures of unidentified bacteria have now been isolated from fresh and dried canaigre roots and from soil in the proximity of growing roots which are able to decompose almost all the sugar in canaigre liquors without materially affecting the tannin. Fermented liquors, after clarification, have been concentrated to powdered extracts having 62 to 65 per cent tannin, not more than 3.5 per cent insolubles, and purities of 65 to 70. Such extracts compare favorably with many from other sources now in commercial use and should be suitable for tanning firm, heavy leather.

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Discussion

C. G. Telander: I have no quarrel with this paper whatsoever. I think the work has been very carefully prepared, so carefully that I do not think there are many questions to be asked. I think the authors are fortunate in having found such friendly bacteria that will ferment the non tannins and not touch the tannins. In the tannery we find the same condition daily but unfortunately we seem to lose tannin also, and we have to put up with it, or, I should rather say, we are allowing it.

I can only throw the discussion open to the floor now, if there are any questions to be asked.

T. F. Oberlander: The first question which comes to my mind is why these large amounts of sugars cannot be used as a useful acid in the tanning operation.

T. C. Cordon: If you were able to ferment all of the sugars to an acid—for instance lactic acid—I doubt if you would be able to prepare suitable extracts because of the large amounts of acid present. Organisms which are found in tan liquors, such as *lactobacilli*, have not been able to ferment canaigre liquor. I don't know whether that will show up in the tanning operation because here we have quite a different situation.